Nanoparticle Based Targeting Approaches for Lung Cancer: A Mini Review

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Abstract — Every year more than 10 million people are diagnosed with cancer and more than 6 million deaths — around 12% of deaths worldwide. The objective of cancer treatment still is to cure the patients if possible or prolong their life and improve their quality of life by surgical removal of the cancerous tissue, radiation therapy, biotherapy, chemotherapy, bone marrow transplant or a combination of some of these available treatments. This review aims to explore the various approaches using nanotechnology and surface decoration with ligands capable to target lung cancers.

Keywords — Cancer, targeting, nanoparticles, lungs, chemotherapy.

I. INTRODUCTION

1.1 Cancer chemotherapy and controlled drug delivery

Cancer is any malignant growth caused by abnormal or uncontrolled cell division eventually spreading to other parts of the body through the lymphatic or the circulatory system [90]. Every year more than 10 million people are diagnosed with cancer and more than 6 million deaths — around 12% of deaths worldwide [1]. It has been estimated that there will be 17 million new cases of cancer every year by 2027. The objective of cancer treatment still is to cure the patients if possible or prolong their life and improve their quality of life by surgical removal of the cancerous tissue, radiation therapy, biotherapy, chemotherapy, bone marrow transplant or a combination of some of these available treatments [2]. It is estimated that more than 50% of the patients of cancer receive systemic chemotherapy as a part of their treatment regimen [3].

Chemotherapy is the use of chemical for treatment or it can be defined as “curing by chemicals” [4]. During chemotherapy, cycles of drugs are prescribed, at interval of three to four weeks, over a treatment period of four to six months. During these cycles, the normal cells (blue line) recover whereas the tumor cells (red line) do not (Fig. 1). Though highly effective in curing the disease, chemotherapy by the use of anticancer drugs possess a lot of adverse effects and has a number of disadvantages.

Fig. 1 Chemotherapy cycles [4]
The main disadvantage of chemotherapy is that normal cells, especially those that divide quickly like bone marrow, lining of gastrointestinal tract or the hair follicles, are also harmed by the anticancer drugs [5].

An effective and advantageous alternative to the conventional chemotherapy is the use of controlled drug delivery systems thereby attaining the release of drug in a predesigned manner from the carrier material [6]. A controlled drug delivery system can improve the efficacy of the drug by rendering sustained and effective drug release (Fig. 2) and also reduce the toxicity and side effects of the anticancer drug [7]. It provides a sustained and effective drug level and improved patient compliance and convenience.

The chemotherapeutic agents most frequently used in NSCLC are cisplatin, paclitaxel, carboplatin, docetaxel, vinorelbine and vinblastin. In cases of advanced lung cancers, targeted therapy drugs such as bevacizumab, and cetuximab can be included in the treatment paradigm. The targeted therapies used in the treatment of NSCLC are designed to inhibit tumor angioneogenesis; inhibit the epidermal growth factor receptor (EGFR) or the ALK gene [10].

### 1.3 Targeted drug delivery systems for lung cancers

The past two decades have been witnessing a lot of research being directed towards targeting of drug molecules to the desired site of action using novel drug delivery systems like nanoparticles. A Pubmed search using “nanoparticles for drug delivery” as the key entry revealed 15266 entries from 2003 to 2023 whereas only 15964 entries from 1990 to 2013. Nanoparticles can be administered via different routes such as oral, parenteral or by pulmonary inhalation. Aerosol therapy [11] with the use of particulate drug carriers is gaining popularity these days as evident from the development of inhalable insulin [12].

The lungs constitute the largest external surface area in the human body, which facilitates an efficient exchange of gases between the blood stream and air. The large alveolar surface area, the low thickness of the epithelial barrier, extensive vascularization and a comparatively lower proteolytic activity in the alveoli and the absence of a first-pass metabolism phenomenon [13] helps in increasing drug absorption and the particulate carrier delivery to the lungs. Nanoparticle delivery to lungs can lead to retention of the particles in the lungs thereby causing a prolonged drug release [14] and studies have shown that particle uptake by alveolar macrophages can be reduced if the particle size is smaller than 260 nm [15].

Several drug delivery systems capable of selectively localizing the drug in the lungs have been developed. The use of external ligands like antibodies, aptamers, proteins, peptides and other small molecule as ligands attached to the nanoparticle carriers results in targeted delivery [16].

The molecular targets that can be used for targeted therapy to the lungs and the various ligand...
conjugated delivery systems that have been developed for targeted delivery have been reviewed in this chapter. In addition to lung targeting, a few other novel delivery systems based on nanocarriers have been reviewed.

1.4 Nanoparticle targeting to lungs after intravenous injection

Initial studies performed by different researchers to investigate the biodistribution of nanoparticles after iv injection showed that the main sites of accumulation of the nanoparticles were the organs of the reticuloendothelial system such as liver, spleen and the lungs [17].

A study by Kreuter et al [18] using poly (methyl-2-14C-methacrylate) nanoparticles injected intravenously in mice and rats and measuring the radioactivity in different organs at different time intervals revealed that the maximum accumulation of the nanoparticles in lungs (21.8% of the administered dose) was after 30 min, which eventually decreased to 13.2% after 7 days of administration of the nanoparticles. Similar distribution studies using different nanoparticles also confirmed that the nanoparticles were taken by the phagocytes of the RES and mainly distributed to the liver and in small portions to the lungs, spleen and the bone marrow [19]. Their study led to a generalization that for the passive targeting of nanoparticles to lungs, the particle size should be more than 7 µm so that the particles are retained in the alveolar capillaries. This occurs due to the breakdown of the repulsive forces of the nanoparticles after injection and their spontaneous agglomeration to form larger particles.

The microspheric particles with sizes ranging from 7-15 µm have been studied by Huo et al [20], Tao et al [21], Ozkan et al [22] and Eldem et al [23]. The findings of their studies indicate that the particles of 7-15 µm size range were able to get retained by the alveolar capillary barrier for longer period of time and thereafter taken up by the alveolar macrophages. The results of study by Eldem et al also cautioned that the particles with diameter of greater than 5 µm may lead to the blockade of blood capillaries and may cause chronic obstructive pulmonary emphysema. Zhang et al [24] reported a HDL-mimicking peptide-phospholipid scaffold (HPPS) nanocarrier for the delivery of diagnostic and therapeutic agents. In their study apoA-I protein of the plasma derived HDL was replaced with self-assembled apoA-I mimetic peptides on the nanoparticle surface. The resulting nanoparticles had a well-controlled size of less than 30 nm and they retained the HDL-like properties to carry the lipophilic payloads. HPPS was also found to mimic the pharmacokinetic properties and targeting specificity of apoA-I protein against scavenger receptor type B1 thereby permitting excellent delivery of the nanoparticles to the target cells.

A study performed by Gipps et al [25] using 14C-polyhexyl cyanoacrylate nanoparticles injected intravenously in nude mice, inoculated with human osteocarcinoma showed that the maximum of 2.27% of the administered dose was accumulated in lungs after 1 h of administration. During a 14 day observation period, this level decreased to 1.11%; the highest levels of nanoparticle accumulation were found in the organs of the RES in their study. Kellaway and Farr [26] tested the possibility of liposomes as drug delivery systems for lung-targeting. Their findings suggested that large particles and liposomes with positive surface charge could be trapped in the capillary beds of the lungs. Waser et al [27] used liposomes, 14C-hexylcyanoacrylate nanoparticles and 125I-albumin nanoparticles to demonstrate that after intravenous injection, the particles could be accumulated in lungs in relatively high concentrations.

Leucata et al [28] compared the pharmacokinetic profile of epirubicin loaded nanoparticles, liposomes and free epirubicin following an intravenous administration of the preparations. A higher level of nanoparticles and liposomes could be found in the lungs but the half life was not significantly increased and the accumulation in lungs was reported to be unimproved in relation to the free drug. In a study initiated by Zara et al [29] on solid lipid nanoparticles loaded with doxorubicin, the concentration of nanoparticles in lungs followed by intravenous injection was found to be much higher compared to the doxorubicin solution. Following an intravenous injection of 14C-azidothymidine attached to hexylcyanoacrylate nanoparticle drugs, Lobenberg et al [30] found that the concentration of Azidothymidine bound to nanoparticles in the lungs compared to control solution was 18 times higher.
after 8 h of administration. While studying the biodistribution of methotrexate-loaded bovine serum albumin nanoparticles, Santhi et al reported 33.14% drug increase in lungs using the nanoparticles compared to the free drug [31]. Solid lipid nanoparticles loaded with dexamethasone acetate developed by Huang et al [32] using high pressure homogenization method and administered intravenously revealed a 17.8 fold increase in the concentration of SLNs in the lungs as compared to dexamethasone solution after 30 minutes of administration.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Lung accumulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (methyl-2-14C-methylacrylate)</td>
<td>21.8%, 30 min after injection and 13.2% after 7 days of injection</td>
<td>[18]</td>
</tr>
<tr>
<td>14C-polyhydroxyl cyanoacrylate</td>
<td>2.27%, 1 h after administration</td>
<td>[15]</td>
</tr>
<tr>
<td>Hexylcyanoacrylate and albumin nanoparticles</td>
<td>Higher accumulation relative to the control solution</td>
<td>[17]</td>
</tr>
<tr>
<td>14C-polylactic acid (PLA) nanoparticles</td>
<td>Negligible after day 1 and 7</td>
<td>[33]</td>
</tr>
<tr>
<td>14C-amino-modified polystyrene</td>
<td>Less than 1% at 2 min after injection</td>
<td>[34]</td>
</tr>
<tr>
<td>Polyhexylcyanoacrylate</td>
<td>Negligible after 6, 18 and 24 h of administration</td>
<td>[35]</td>
</tr>
<tr>
<td>Poly (methyl methacrylate)</td>
<td>No improved accumulation compared to control solution</td>
<td>[36]</td>
</tr>
<tr>
<td>Polybutylcyanoacrylate</td>
<td>Lower drug concentration relative to free drug solution</td>
<td>[37]</td>
</tr>
<tr>
<td>Gelatin</td>
<td>No significant change in accumulation as compared to free drug solution</td>
<td>[38]</td>
</tr>
<tr>
<td>Solid lipid nanoparticles</td>
<td>Higher concentration compared to control solution</td>
<td>[29]</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>33.14% increase compared to free drug</td>
<td>[311]</td>
</tr>
<tr>
<td>Hexylcyanoacrylate</td>
<td>18 times drug concentration increase in lungs compared to control solution</td>
<td>[30]</td>
</tr>
<tr>
<td>Surfactant-coated polymethyl [2-14C] Methacrylate</td>
<td>7%, 24 h after administration</td>
<td>[38]</td>
</tr>
<tr>
<td>Albumin</td>
<td>Significant tumor response</td>
<td>[39]</td>
</tr>
</tbody>
</table>

### 1.5 Ligands for targeting lungs

The use of ligands attached along with the carrier can be helpful in bioadhesion, cell uptake or transcytosis of the delivery system thereby increasing the targeting efficiency of the delivery system. The development of molecular biology, proteomics and combinatorial approaches has led to the screening of more and more targeting ligands. Some of the ligands proven to be effective in targeting of the particulate delivery systems to lungs are given in Table 2.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Receptor</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin</td>
<td>Lectin receptor</td>
<td>Improved uptake of drugs and liposomes into airway cell</td>
<td>[41]</td>
</tr>
<tr>
<td>Sugars</td>
<td>Lectin receptors</td>
<td>More efficient uptake by airway epithelial cells in culture</td>
<td>[42, 43]</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------</td>
<td>------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Alveolar macrophage receptors</td>
<td>Specific, enhanced endocytic uptake into alveolar macrophages</td>
<td>[44, 45]</td>
</tr>
<tr>
<td>Immuno-globulins</td>
<td>IgG</td>
<td></td>
<td>[46, 47]</td>
</tr>
<tr>
<td>Lipo-proteins</td>
<td>SP-A</td>
<td>Increased drug and liposome delivery to airway cells</td>
<td>[48-50]</td>
</tr>
<tr>
<td>EGF</td>
<td>rEGF</td>
<td>Efficient DNA uptake into cancer cells</td>
<td>[51-53]</td>
</tr>
<tr>
<td>Mono-clonal Abs</td>
<td>ICO-25 Mab</td>
<td>Specific delivery to tumor cells of epithelial origin</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>Anti-ICAM-1 antibodies</td>
<td>IFN-gamma activated human bronchial epithelial cells (BEAS-2B)</td>
<td>[55]</td>
</tr>
<tr>
<td>Peptides</td>
<td>THALWHT</td>
<td>Specific binding and uptake into human airway epithelial cells in vitro</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>linear/cyclic PLAEIGIEL</td>
<td>Efficient DNA delivery to airway cells in culture</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td>RGD</td>
<td>Increased gene transfer Efficiency</td>
<td>[58]</td>
</tr>
<tr>
<td>Receptor agonists/ antagonists</td>
<td>UTP</td>
<td>Efficient gene transfer in human airway cells</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>Folate</td>
<td>Efficient internalization into FR-expressing murine lung carcinoma cell line</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>Transferrin</td>
<td>Improved uptake into alveolar epithelial cells</td>
<td>[61]</td>
</tr>
</tbody>
</table>

IGg, immunoglobulin; rEGF, recombinant epidermal growth factor; SP-A, surfactant protein A; RGD (peptide sequence); PLAEIGIEL (peptide sequence); Uridine 5’Triphosphate (UTP).

1.6 Lung Vascular Receptors

More than 40 different cell types are known to be present as components of the human lung vascular bed and respiratory airways [62]. Techniques like phage display, cDNA array and cell culture methods have been helpful in identifying some potential receptors in the lung vasculature that may be targeted for an effective drug delivery (Table 3).
Table 3 Some preferentially expressed receptors in lung vasculature [63]

<table>
<thead>
<tr>
<th>Lung vasculature receptor</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Identified using cell culture methods</strong></td>
<td></td>
</tr>
<tr>
<td>α-galactose and α-N-actyl-galactosamine</td>
<td>Microvascular endothelial cells</td>
</tr>
<tr>
<td>α- and β-N-actyl-galactosamine</td>
<td>Pulmonary artery derived endothelial cells</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Microvascular endothelial cells (not expressed by artery endothelial cells)</td>
</tr>
<tr>
<td><strong>Identified using Phage display</strong></td>
<td></td>
</tr>
<tr>
<td>Membrane dipeptidyl peptidase (CD26)</td>
<td>Lung endothelial cells</td>
</tr>
<tr>
<td><strong>Identified using cDNA array</strong></td>
<td></td>
</tr>
<tr>
<td>Phospholipase A2 group XII</td>
<td>Lung endothelial cells</td>
</tr>
<tr>
<td>Secreted frizzled related protein 1 (sFRP1)</td>
<td>Lung endothelial cells</td>
</tr>
<tr>
<td>Osteoglycin</td>
<td>Lung endothelial cell, smooth muscle cell, around cartilage and alveoli</td>
</tr>
<tr>
<td><strong>Using other approaches</strong></td>
<td></td>
</tr>
<tr>
<td>Ca²⁺-activated chloride channels (human CLCA-2/mouse CLCA-1/Lu-ECAM-1)</td>
<td>Endothelia of the aorta and pulmonary venules</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV (CD26)</td>
<td>Lung endothelium</td>
</tr>
<tr>
<td>Angiotensin converting enzyme (ACE) (lung selective marker)</td>
<td>Endothelial luminal surface</td>
</tr>
<tr>
<td>Platelet-endothelial adhesion molecule-1 (PECAM-1)/CD31 (lung selective marker)</td>
<td>Intercellular borders of the endothelial monolayer</td>
</tr>
<tr>
<td>Aminopeptidase P (APP)</td>
<td>Caveola of lung endothelium</td>
</tr>
</tbody>
</table>

1.7 Some nanoparticulate delivery systems used in cancer therapy

A few nanoparticulate delivery systems for anticancer molecules have also been reviewed for the purpose of designing novel polymeric system for drug targeting. Cheng et al [64] reported nanoparticle-aptamer bioconjugates for in vivo cancer therapy. Using prostate cancer as a model, they reported docetaxel encapsulated nanoparticles formulated with biocompatible and biodegradable PLGA-b-PEG copolymer surface functionalized with the A10 2'-fluoropyrimidine RNA aptamers that recognize the extracellular domain of the prostate-specific membrane antigen (PSMA), a well characterized antigen expressed on the surface of prostate cancer cells.

Reddy and Murthy [65] developed etoposide loaded nanoparticles using melt- emulsification and homogenization followed by spray drying of nanodispersion. Functionalized polymeric nanoparticles have been synthesized by an emulsifier-free emulsion polymerization by Lepoittevin et al [66]. They used two monomers for polymerization: 2-aminoethylmethacrylate and 4-(2-aminoethylthio) methylstyrene. Various glucose molecules such as maltose, galactopyranosyl ethanol lactose, and maltotriose were covalently grafted on to the surface of the nanoparticles by reductive amination or amidation.

Lee et al [67] formulated nanoparticles of poly (lactide)-tocopheryl polyethylene glycol succinate copolymer for protein drug delivery. Compared with
other biodegradable polymeric nanoparticles such as poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles, the PLA-TPGS nanoparticles were able to provide the encapsulated proteins with a milder environment. Confocal laser scanning microscopy (CLSM) observation demonstrated the intracellular uptake of the PLA-TPGS NPs by NIH-3T3 fibroblast cells and Caco-2 cancer cells. A study by Gao et al [68] on novel DOCA modified CM-curdlan (DCMC) conjugate self-assembled nanoparticles loaded with epirubicin exhibited drug release in sustained manner. The in vitro anti-tumor studies using MCF-7 cells showed that the nanoparticles were more cytotoxic than the drug alone, which was due to the higher uptake of the nanoparticles in tumor. In vivo studies indicated that DCMC conjugate did not cause unexpected side effects. A study was conducted by Paranjape et al [69] to design tumor-targeted bioconjugate based delivery of camptothecin. A novel CPT bioconjugate was synthesized using carbodiimide chemistry with a linear PEG and amino acid glycine, respectively as the spacer and linker. Folic acid was used as the ligand for targeting taking into consideration the presence of mechanism for folate receptor mediated endocytosis. The results indicated significantly higher efficacy of the bioconjugate in comparison to CPT. In an investigation by Jain et al [70] on tumor targeted doxorubicin loaded surface tailored solid lipid nanoparticles (SLNs) conjugated with mannose, it was found that the biodistribution of drug from mannosylated SLNs was higher in the tumor mass as compared to the non-mannosylated SLNs. Chandna et al [71] compared the influence of different characteristics of nanocarriers on the efficacy of chemotherapy and imaging. It was evident from the results of their study that targeting to cancer cells by LHRH peptide enhanced antitumor activity of all tested nanocarriers. Park et al [72] prepared Adriamycin encapsulated nanoparticles using deoxycholic acid conjugated dextran and evaluated its antitumor activity using CT 26 tumor cells in vitro and in vivo. The nanoparticles were found to be promising vehicles for antitumor drug delivery. In another study Park et al [73] reported a delivery system comprising of modified heparin-DOCA conjugate as drug carrier for cancer therapy. The conjugate was able to prevent squamous cell carcinoma and human umbilical vascular endothelial cell proliferation during bromodeoxyuridine (BrdU) incorporation assays.

Bozkir and Saka [74] reported the formulation of 5-FU nanoparticles using factorial design studies. They used the approach of an orthogonal experimental design to optimize the formulation of 5-fluorouracil (5-FU) loaded poly D,L (lactide-co-glycolide) (PLGA) nanoparticles (5FU-NP) by a nanoprecipitation-solvent displacement method. Maghsoudi et al [75] studied bovine serum albumin (BSA) nanoparticles loaded with 5-fluorouracil. The BSA nanoparticles suspension was found to maintain a constant release of drug for 20 h under experimental conditions and hence was found to be capable of releasing drug in a sustained manner over prolonged time period. Zhang et al [76], studied the anti-carcinoma effect of chitosan-polyaspartic acid-5-fluorouracil nanoparticle on tumor growth in nude mice. The tumor inhibition rates for the CTS-Pasp, 5-FU and CTS-Pasp-5FU groups were 5.09%, 65.3% and 72.79%, respectively. Yassin et al [77] performed a study using 5-FU solid lipid nanoparticles consisting of triglycerides esters, DynasanTM 114 or DynasanTM 118 along with soya lecithin as the lipid parts to treat colon cancer. The results exhibited a delayed release of the loaded drug in simulated colonic medium containing rat caecal contents.

Nanoparticles of particular sizes are known to have capacity to penetrate the BBB. The ability of such nanoparticles to cross the BBB has been utilized for targeted delivery of drugs to the CNS by Tosi et al [78]. They derivatized PLA with g7 peptide and the resulting g7 nanoparticles were loaded with loperamide and rhodamine-123 in order to assess their ability as drug carriers to the CNS. A study reported by Cho et al [79] showed the targeting capability of surface modified trans-retinoic acid (RA) loaded PLA nanoparticles. The study was carried out by using galactose as the hepatocyte-specific targeting ligand. It was found that the shapes of most hepatocytes attached onto polystyrene dish precoated with collagen solution were flat and spreading at low concentration of RA for the RA-loaded nanoparticles, whereas their shapes were round at even low concentration of RA when RA was mixed with the nanoparticles. De-Hong Yu et al [80] investigated K237-(HTMYHYHQHHL) peptide-
conjugated biodegradable nanoparticles as a carrier to target paclitaxel to tumor vasculature. The K237 conjugated nanoparticles were found to be significantly internalized by human umbilical vein endothelial cells (HUVEC) through the K237-KDR interaction, and this facilitated uptake was considered to be responsible for the enhanced antiangiogenic activity shown by HUVEC proliferation, migration and tube formation compared to cells treated with the commercial formulation Taxol® and PTX-NP.

Dubey et al [81] in their study performed biological evaluation of paclitaxel loaded biodegradable PCL/PEG nanoparticles for the treatment of human neuroendocrine pancreatic tumor in mice. The PCL-Ptx nanoparticles reduced tumor volume significantly in comparison with paclitaxel. A biphasic release pattern was obtained for Paclitaxel-loaded PEGylated PLGA-based nanoparticles by Danhier et al [82]. The in vitro anti-tumoral activity was assessed using the Human Cervix Carcinoma cells (HeLa) by the MTT test relative to the commercial formulations Taxol® and Cremophor® EL. PtX-loaded nanoparticles showed greater tumor growth inhibition effect in vivo on TLT tumor as compared to Taxol®. Kou et al [83] investigated paclitaxel-loaded PLGA nanoparticles coated with cationic SM5-1 single chain antibody. PTX-NP-S was shown to retain the specific antigen-binding affinity of SMFv-polylys to SM5-1 binding protein-positive Ch-hep-3 cells. The cytotoxicity of PTX-NP-S was also evaluated by a non-radioactive cell proliferation assay. It was evident from the results that PTX-NP-S had significantly enhanced in vitro cytotoxicity against Ch-hep-3 cells as compared with non-targeted paclitaxel-loaded PLGA nanoparticles.

II.  CONCLUSION

The objective of this review was to explore various nanotechnology based approached used to target drug delivery to lungs for treatment of lung cancers. It could be concluded from the review that the use of ligand as surface decorations has enabled a plethora of domains and portals to channelize the localization of drug in higher amounts to the desired organ or tissue.

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